AD_____

Award Number: W81XWH-06-1-0108

TITLE: Stromal Mesenchyme Cell Genes in Prostate Cancer Development: Epigenetic Markers for Cancer and Potential Targets for Therapy

PRINCIPAL INVESTIGATOR: Young Ah Goo, Ph.D.

CONTRACTING ORGANIZATION: University of Washington
Seattle WA 98105

REPORT DATE: December 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-12-2006	Annual	12 Nov 05 – 14 Nov 06

4. TITLE AND SUBTITLE
Stromal Mesenchyme Cell Genes in Prostate Cancer Development: Epigenetic Markers for Cancer and Potential Targets for Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0108

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Young Ah Goo, Ph.D.

E-Mail: youngah@u.washington.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Washington
Seattle WA 98105

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Functional development of the prostate is governed by stromal induction and epithelial response. Stromal/epithelial signaling can be mediated through direct cell-cell contact and diffusible factors with their cell surface receptors. One approach to gain a molecular understanding of epithelial/stromal interaction is to identify the organ-specific stromal signaling factors. We proposed to do this through a comparative analysis between prostate stromal and bladder stromal cells. For the prostate/bladder transcriptome analysis, we isolated stromal cell populations by the use of specific CD markers: CD49a for prostate and CD13 for bladder with magnetic cell sorting (MACS) and laser-capture microdissection (LCM). The Affymetrix microarray platform was used to determine the cell type-specific transcriptomes. The glycopeptide-capture method was used to profile protein expression differences because most secreted proteins are glycosylated. We have identified an increased expression in prostate stromal cells of signal transducers like AXL receptor tyrosine kinase, and protein tyrosine kinase 7, which have been implicated in tumor progression. Western blot analysis was performed to validate their expression. Our comparative stable isotope-free proteomic analysis using a modification of the N-linked glycopeptide-capture method allowed facile measurement of changes in protein expression. A number of secreted proteins which may be involved in stromal/epithelial signaling and organ-specific differentiation were identified.

15. SUBJECT TERMS
stromal/epithelial interaction, stromal signaling molecules, prostate differentiation, microarray gene expression analysis, glycopeptide-capture method, mass spectrometry

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION

The prostate develops from the urogenital sinus, and this development is governed by stromal induction and epithelial response. Stromal/epithelial signaling can be mediated through direct cell-cell contact and diffusible factors and their cell surface receptors. Given the importance of intercellular communication, it is possible that diseases such as benign prostatic hyperplasia and cancer could arise from a defect in or a loss of this communication. One approach to gain a molecular understanding of epithelial/stromal interaction is to identify, as a first step, the organ-specific stromal signaling factors. We proposed to identify genes responsible for the organ-specific induction of epithelial differentiation through a comparative transcriptome analysis between sorted prostate stromal and bladder stromal cells. Furthermore, stromal signaling factors are made when the stromal cells are grown in culture, since epithelial induction can be achieved with stromal cell conditioned media. As such, quantitative proteomic analysis was carried out to identify differentially expressed secreted proteins found in the culture media of prostate and bladder stromal cells.

BODY

A. Identification of differentially expressed stromal mesenchyme cell genes between the prostate and bladder using isolated cell populations

A.1. Isolation of single cell populations by their specific CD markers using a bulk isolation method with antibody-coated magnetic beads (MACS)

Our CD phenotyping result showed a layer of stromal cells beneath the bladder urothelium that was positive for CD13 whereas the stromal cells next to the prostatic epithelium were not (Fig. 1). This comparative analysis was therefore expected to reveal differences in gene expression between the two stromal cell types, and most, if not all, of the differentially expressed genes that might play a role in the prostate or bladder differentiation program. For comparative analysis, we isolated single cell populations by the use of CD49a for prostate and CD13 for bladder stromal cells.

Prostate and bladder tissue specimens were obtained from Department of Urology tissue acquisition program. For sorting, tissue samples weighing between 1-10 g are minced and digested by collagenase in RPMI1640 supplemented with 5% FBS and 10^{-8} M DHT.[1] The resultant single cells are partitioned on discontinuous Percoll density gradients. Cells banding at the stromal cell density (ρ=1.035) are collected as STROM, and cells banding at the epithelial cell density (ρ=1.07) are collected as EPI. $CD49a^+$ cells are sorted by MACS from STROM. $CD13^+$ cells are sorted from bladder STROM. In MACS, cells are resuspended in 0.1% BSA-HBSS and labeled with the appropriate phycoerythrin (PE)-conjugated antibodies. After the primary antibody, the cells are incubated with anti-PE microbeads. The cell suspension are filtered and sorted by AutoMACS using the POSITIVE SELECTION DOUBLE SORT program (Miltenyi Biotec). Both the positive and negative fractions are analyzed by flow cytometry to assess purity. We use MACS because of the shorter sort time (~30 min) compared to FACS (Fluorescence Activated Cell Sorting) in order not to compromise cell viability. The sorted cells are lysed for RNA preparation and the RNA quality is checked by BioAnalyzer (Aglient technologies).

This step has accomplished Statement of Work: Task 1.a. Isolate single cell populations by their specific CD markers using a bulk isolation method with antibody-coated magnetic beads (MACS)

A.2. Isolation of stromal cells by laser-capture microdissection (LCM)

For LCM, eight μm thick sections of frozen tissue blocks were prepared, immediately fixed in cold 95% ethanol, briefly stained with hematoxylin using Arcturus HistoGene Staining Solution and dehydrated in 100% ethanol followed by xylene, as described in the Arcturus HistoGene LCM Frozen Section Staining Kit protocol. Around 5,000 stromal cells were captured using Arcturus PixCell II. Following microdissection, captured cells were lysed in Arcturus RNA Extraction Buffer. RNA was isolated using Arcturus PicoPure RNA Isolation and treated with DNase. The RNA was amplified by two rounds using Ambion MessageAmp aRNA. RNA sample quality and quantification were assessed by BioAnalyzer.

This step has accomplished Statement of Work: Task 1.b. Isolate stromal cells by laser-capture microdissection (LCM)

A.3. Array analysis of the sorted cells by Affymetrix oligonucleotide microarray

Gene expression was profiled by the Affymetrix human gene-chip arrays. The UG-133 Plus 2.0 gene chip contains probe sets representing 54,675 genes, splice variants, and ESTs. Analyzed were: $CD49^+$ (prostate stromal) cells, vs. $CD13^+$ (bladder stromal) cells; prostate LCM stromal cells, vs. bladder LCM stromal cells. In using the arrays, total RNA was reverse transcribed with a poly-T primer/T7 promoter to produce cDNA. Second-strand cDNA was then synthesized. An in vitro transcription is performed in the presence of biotinylated ribonucleotides. The labeled cRNA was hybridized to the arrays, washed and stained with streptavidin-PE using Affymetrix FS-450 fluidics station, and data is collected with GeneChip Scanner 3000. The Affymetrix data was analyzed with GeneChip Operating Software (GCOS). Scanned images of the arrays were converted to numerical data by GCOS and outputted to tab delimited text files containing Affymetrix probeset ID, signal, present or absent detection call, and detection P-value fields for each probe set. The raw Affymetrix data was filtered to mask genes with signal intensities greater the background threshold. The raw data was further filtered by retaining only genes that are called present by GCOS in both replicates for each source material. Finally an analysis was performed on the filtered data across other datasets to determine which genes are differentially expressed in the comparison.

Preparation of high-quality RNA from the sorted or LCM cells were technically challenging. We exercised great caution during the entire tissue processing including the cell sorting in order not to compromise cell viability. A successful cell sorting is based on combination of unknown factors including quality and weight of starting tissue. Task 1 has been slightly delayed due to the above-mentioned technical difficulty. However, enough microarray experiments have been completed. Data analysis and validation will be completed in time.

This step has accomplished experimental parts of the Statement of Work: Task 1.C. Analyze by DNA arrays of isolated cells for cell type-specific transcriptomes. Complete analysis of microarray data is currently under progress

B. Identification of differentially expressed secreted proteins of stromal cells by quantitative proteomic analysis

B.1. Prostate and bladder stromal cell culture and cell-free conditioned media preparation

Cultures were started either by placing cut tissue pieces on tissue culture plates or by plating single cells prepared by tissue digestion with collagenase as described previously.[2] Prostate or bladder stromal cells from Percoll gradients were cultured in RPMI-1640 supplemented with 10% FBS and 10^{-8} M DHT. Cells were serially passaged. For identity, the cells were checked for non-expression of epithelial cytokeratins and epithelial cell-specific tyrosine kinase *c*-MET by immunocytochemistry and RT-PCR[3] respectively. Prostate and bladder stromal cells at the third or fourth passage were placed in RPMI-1640 without FBS and cultured for 24 h. The time was determined based on our previous experiments not to produce serum-shock proteins (unpublished data). Serum-free media was used because FBS would overwhelm the proteomic analysis with bovine albumin and other highly abundant proteins. The cell-free media was prepared by centrifugation and filtration. The proteins in media were concentrated by using Centricon Plus-20 (NMWL: 5000, Millipore, Billerica, MA). The tissue digestion media was also analyzed.

This step has accomplished Statement of Work: Task 2.a. Culture prostate and bladder stromal cells in vitro and prepare cell-free conditioned media

B.2. Identification of differentially expressed secreted glycoproteins

Secreted proteins of prostate and bladder stromal cells were captured by using *N*-linked glycopeptide-capture method.[4] Briefly, 1 mg proteins from two biological samples were added to 100 mM NaAc and 150 mM NaCl, pH 5.5 in a desalting column (Bio-Rad, Hercules, CA) and oxidized at room temperature for 1 h by adding 15 mM sodium periodate. After removal of the oxidant, the sample was conjugated to hydrazide resin (Bio-Rad, Hercules, CA) at room temperature for 10–24 h. Non-glycosylated proteins were then removed by washing the resin three times with 1 ml 8 M urea, 0.4 m NH_4HCO_3, pH 8.3. After the last wash, proteins were reduced by 8 mM Tris (2-carboxyethyl) phosphine at room temperature for 30 min and alkylated at room temperature for 30 min by 10 mM iodoacetamide. The resins were washed twice with 1 ml 0.1 M NH_4HCO_3. Afterwards, the resin was resuspended in 0.1 M NH_4HCO_3 and trypsin was added at 1 mg/200 mg of protein and digested at 37 °C overnight. The trypsin-released peptides were removed, and the resin was washed three times each with 1.5 M NaCl, 80% acetonitrile, 100% methanol, water, and 0.1 M NH_4HCO_3. *N*-Linked glycopeptides were released from the resin by 0.3 µl of peptide-N-glycosidase F (New England Biolabs, Beverly, MA) in 0.3 ml 0.1 M NH_4HCO_3 and incubation at 37 °C overnight. The released peptides were dried and resuspended in 50% acetonitrile, 0.1 % formic acid for mass spectrometry.

Glycopeptides were fractionated by online-reverse phase cation-exchange chromatography and each fraction was analyzed by tandem mass spectrometry. The PeptideProphet and ProteinProphet,[5] which computes a probability for the likelihood of each identification being correct, were used for statistical analysis. PeptideProphet probability score of ≥ 0.5 was used as a filter with an error rate of 3% or less. ProteinProphet probability score of ≥ 0.9 with less than 0.02% error rate of was used. In order to reduce the false positive rate, peptides were further filtered for N-X-S/T (X is any amino acid except proline) motif. The preliminary study showed identification of a smaller number of proteins (presumably due to more complex sample dynamic range) and increased detection of intracellular proteins from the tissue digestion media than the cell-free media of cultured cells. Since the purpose of the study is to identify secreted proteins, future investigation will concentrate on using cell-free conditioned media.

Protein expression was estimated by a novel isoptope-free quantification software developed in-house (unpublished data). Briefly, the expression levels of protein were estimated by using peak intensity area.[6,7] After background noise subtraction, the peak intensities were normalized using total ion intensity. All peptides identified for a given protein were collected, and the theoretical *m*/*z* of first three isotope peaks of a peptide was calculated. Peaks with 3 or more scans to reconstruct an ion chromatogram were extracted in the study. The protein expression was estimated by summing the peak areas of its corresponding peptides. The protein ratio between bladder and prostate was derived by dividing an average protein expression value of bladder by the average protein expression value of prostate. Differentially expressed proteins were identified by two-sample *t*-test (with unequal variance), and a false discovery rate (FDR) was measured to correct for multiple testing variations.

A total of 120 glycoproteins were identified in the media of prostate stromal cell culture. Of those, 34 proteins were identified in the prostate but not the bladder. A total of 88 glycoproteins were identified in the bladder stromal cell culture media and 2 of those were identified in the bladder but not the prostate (Fig. 2). The identified glycoproteins were analyzed by Gene Ontology (GO) (http://www.geneontology.org/). Accordingly, approximately 50% were classed as secreted or membrane-associated cell surface proteins (Fig. 3). More than 75% with known functions were extracellular proteins attesting to the efficiency of the glycopeptide-capture method. These proteins are mostly involved in physiological process, cell adhesion, communication, and development (Fig. 4). Functions such as binding, receptor, and transport could be involved in intercellular communication. Of the total 122 secreted proteins, 33 proteins with false discovery rate ≤ 0.05 were considered to be statistically significant for differential expression, and are listed in Table 1.

This step has accomplished Statement of Work: Task 2.b. and 2.c.

B.3. Validation of differential expression by Western blot

Among the 33 proteins, 25 proteins were over expressed in the prostate and 8 proteins were over expressed in the bladder. Cathepsin L precursor (CTSL) was the most differentially expressed protein in prostate stromal cells while beta-2-glycoprotein I precursor (APOH) was the most differentially expressed protein in bladder stromal cells. Differential expression of these proteins and others was tested by Western blot analysis using specific antibodies (Fig. 5). At least two antibodies from two different vendors were tested if the experiments failed to work

with the first antibody. For some proteins, no antibody was available or the antibody failed to work after repeated trials. Our result showed that the expression of follistatin-related protein 1 (FSTL1), tumor necrosis factor receptor superfamily member 11B (TNFRSFIIB), SPARC, and metalloproteinase inhibitor 1 (TIMP1) were increased in the prostate while beta-2-glycoprotein I (APOH) expression was slightly increased in the bladder. Serotransferrin (TF) and stanniocalcin 1 (STC1) were increased in the prostate. CD90 was used as a control to show equal loading of proteins.

This step has accomplished Statement of Work: Task 2.d.

Task 2 has almost been accomplished ahead of the proposed time line. A manuscript is currently in preparation to summarize this part of study.

KEY RESEARCH ACCOMPLISHMENTS

A. Isolate $CD49a^+$ prostate stromal cells and create prostate stromal cell-specific transcriptomes

B. Isolate $CD13^+$ bladder stromal cells and create bladder stromal cell-specific transcriptomes

C. Identify secreted proteins of prostate stromal cells from the conditioned media by glycopeptide-capture proteomics

D. Identify secreted proteins of bladder stromal cells from the conditioned media by glycopeptide-capture proteomics

E. Identify differentially expressed secreted proteins between prostate and bladder stromal cells and validate differential expression by Western blot analysis

REPORTABLE OUTCOMES

Goo, YA., Ryu, S., Walashek, L., Shaffer, S., Liu, AY., Goodlett, DR. Comparative analysis of secreted proteins of human prostate and bladder stromal mesenchyme cells. 7th Siena Meeting: From Genome to Proteome. Siena, Italy Oct 2006, Poster presentation

Goo, YA., Ryu, S., Shaffer, S., Walashek, L., Liu, AY., Goodlett, DR. Identification of secreted glycoproteins of human prostate and bladder stromal cells by comparative quantitative proteomics analysis. USHUPO 3rd Annual Conference. Seattle, USA Mar 2007, Poster presentation (accepted)

CONCLUSION

We have identified prostate-specific stromal/epithelial signaling molecules through a comparative transcriptome analysis between sorted or laser-captured prostate stromal and bladder stromal cells. Furthermore, most, if not all, of these products are made when stromal cells are in culture, since epithelial induction can be achieved with stromal cell conditioned media. Quantitative proteomic analysis using the glycopeptide-capture method (for secreted proteins) was carried out. Our comparative stable isotope-free proteomic analysis identified a number of secreted proteins which may be involved in stromal/epithelial signaling and organ-specific differentiation.

REFERENCES

1. Liu AY, True LD, LaTray L, Nelson PS, Ellis WJ, Vessella RL, Lange PH, Hood L, van den Engh G: **Cell-cell interaction in prostate gene regulation and cytodifferentiation.** *Proc Natl Acad Sci U S A.* 1997, **94**:10705-10.

2. Liu AY, LaTray L, van den Engh G: **Changes in cell surface molecules associated with in vitro culture of prostatic stromal cells.** *Prostate* 2000, **45**:303-312.

3. Goo YA, Goodlett DR, Pascal LE, Worthington KD, Vessella RL, True LD, Liu AY: **Stromal mesenchyme cell genes of the human prostate and bladder.** *BMC Uro.* 2005, **12**:5:17.

4. Zhang H, Li XJ, Martin DB, Aebersold R: **Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry.** *Nat Biotechnol* 2003, **21**:660-6.

5. Goo YA, Yi EC, Baliga NS, Tao WA, Pan M, Aebersold R, Goodlett DR, Hood L, Ng WV: **Proteomic analysis of an extreme halophilic archaeon, Halobacterium sp. NRC-1.** *Mol Cell Proteomics* 2003, **2**:506-24.

6. Radulovic D, Jelveh S, Ryu S, Hamilton TG, Foss E, Mao Y, Emili A: **Informatics platform for global proteomic profiling and biomarker discovery using liquid chromatography-tandem mass spectrometry.** *Mol Cell Proteomics* 2004, **3**:984-97.

7. Ryu S, Radulovic D, Gallis B, Goo YA, Shaffer SA, Goodlett DR: **Comparison of Isotope Coded Affinity Tag (ICAT) to Regular Old Ordinary Mass Spectrometry (ROOMS) for Quantitative Proteomics.** *2005 ASMS POSTER* ThP32-521.

SUPPORTING DATA

Fig. 1. CD13 immunohistochemistry of the prostate and bladder. CD13 stains luminal epithelial cells (black arrow) of prostatic glands as shown on the left. In the bladder, CD13 stains a subpopulation of stromal cells (black arrow) in the lamina propria as shown on the right. The partially denuded urothelium is indicated by the red arrow. Magnification is 40x.

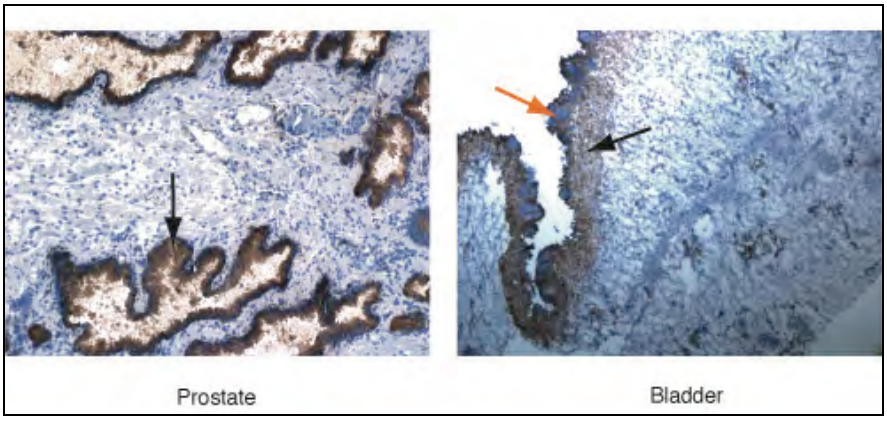

Fig. 2. Number of secreted glycoproteins found in cell culture media of prostate (PS) and bladder (BL) stromal cells

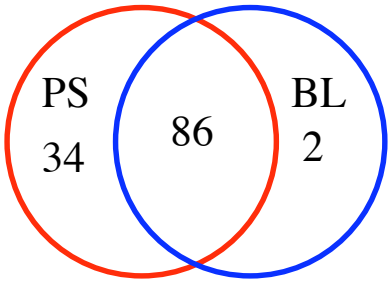

Fig. 3. Cellular compartment distribution

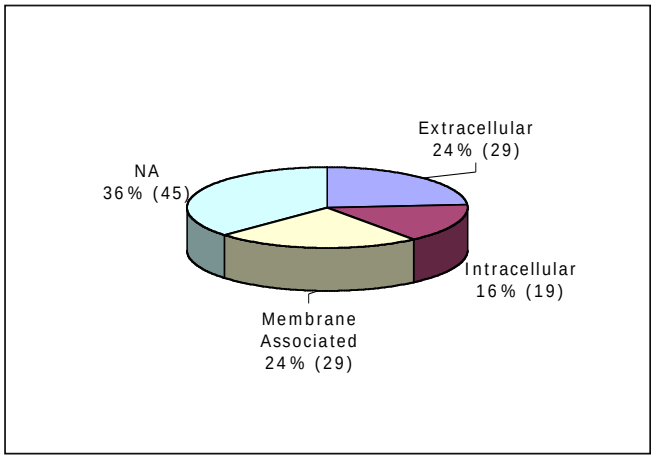

Fig. 4. Functional aspect of secreted stromal proteins

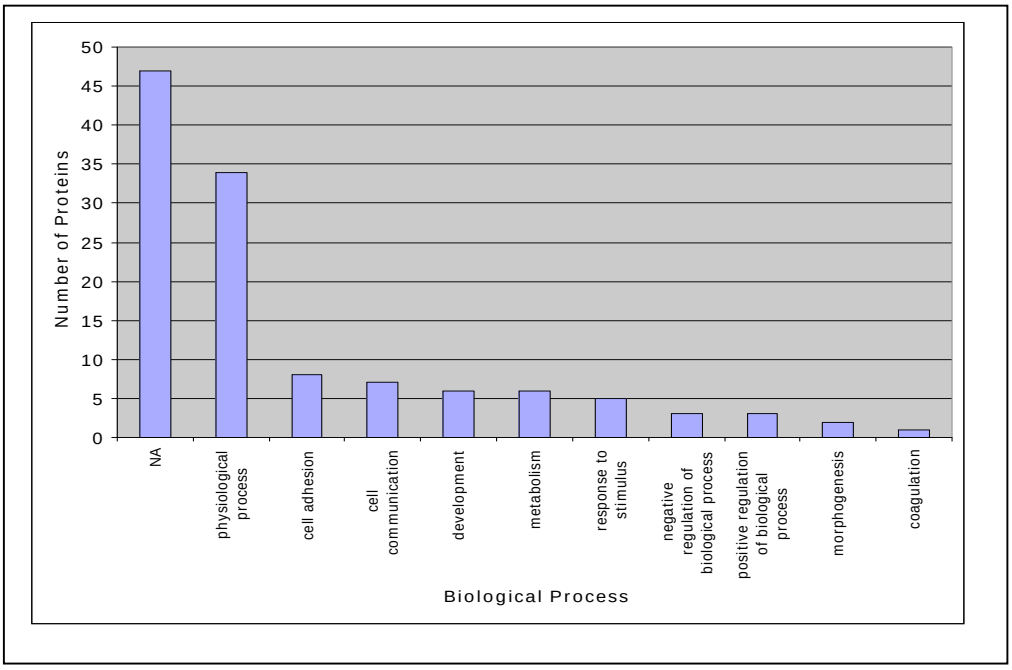

Fig. 5. Validation of differential expression

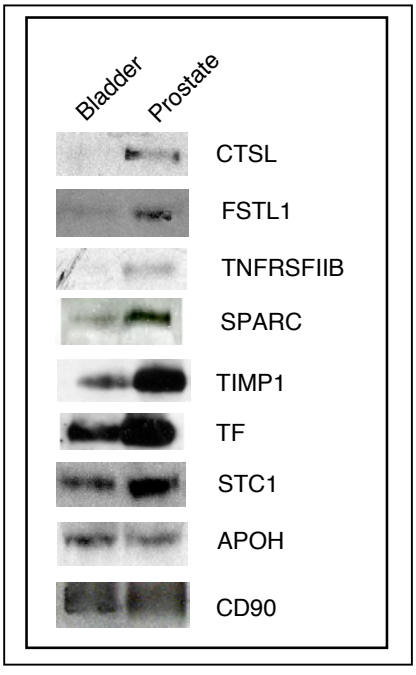

Table 1. Differentially expressed secreted stromal cell proteins

Protein	Probability[+]	Annotation	PS/BL*	False discovery rate**	Peptides***
IPI00012887	1	Cathepsin L precursor	4.9	0.001	YSVA**NDT**GFVDIPKQEK
IPI00029723	1	Follistatin-related protein 1 precursor	11.0	0.001	GS**NYS**EILDK
IPI00297160	1	Hypothetical protein DKFZp451K1918	3.7	0.001	AF**NST**LPTMAQMEK
IPI00029131	1	Neuroendocrine convertase 2 precursor	3.0	0.001	RGDLNI**NMT**SPMGTK
IPI00015657	1	Pregnancy-specific beta-1-glycoprotein 5 precursor	9.9	0.001	ILILPSVTR**NET**GPYECEIR
IPI00298281	1	Laminin gamma-1 chain precursor	7.6	0.001	LLN**NLT**SIK
IPI00020986	1	Lumican precursor	2.6	0.001	LHINHN**NLT**ESVGPLPK
IPI00296992	0.99	AXL receptor tyrosine kinase, isoform 1	7.0	0.002	EESPFVGNPG**NIT**GAR
IPI00298362	1	Tumor necrosis factor receptor superfamily member 11B precursor	3.9	0.002	HIGHA**NLT**FEQLR
IPI00339227	1	Splice Isoform 7 Of Fibronectin precursor	7.5	0.002	DQCIVDDITYNV**NDT**FHK
IPI00014572	1	SPARC precursor	7.2	0.002	VCSND**NKT**FDSSCHFFATK
IPI00009198	1	Tissue factor pathway inhibitor 2 precursor	4.0	0.002	DEGLCSA**NVT**R
IPI00328113	1	Fibrillin 1 precursor	7.4	0.002	TAIFAF**NIS**HVSNK
IPI00018305	0.99	Insulin-like growth factor binding protein 3 precursor	10.9	0.003	GLCV**NAS**AVSR
IPI00419941	1	PTK7 protein tyrosine kinase 7, isoform a	1.7	0.003	MHIFQ**NGS**LVIHDVAPEDSGR
IPI00021081	1	Splice Isoform 1 Of Follistatin precursor	2.2	0.003	SDEPVCASD**NAT**YASECAMK
IPI00032292	1	Metalloproteinase inhibitor 1 precursor	2.1	0.004	SH**NRS**EEFLIAGK
IPI00298828	1	Beta-2-glycoprotein I precursor	0.2	0.006	VYKPSAG**NNS**LYR
IPI00291866	1	Plasma protease C1 inhibitor precursor	0.5	0.006	DTFV**NAS**R
IPI00470937	1	Protein tyrosine phosphatase, receptor type, K	3.8	0.006	GPLANPIW**NVT**GFTGR
IPI00289819	1	Cation-independent mannose-6-phosphate receptor precursor	1.5	0.008	DAGVGFPEYQEED**NST**YNFR
IPI00022810	1	Dipeptidyl-peptidase I precursor	63.9	0.008	DV**NCS**VMGPQEK
IPI00004618	1	IGHG4 protein; Beta-2-microglobulin	0.8	0.010	EEQF**NST**YR
IPI00016112	1	peroxidasin homolog PREDICTED: Melanoma associated gene	10.6	0.012	QGEHLS**NST**SAFSTR
IPI00478097	1	HU-K4 phospholipase D3 isoform 1	0.5	0.015	AALRD**NHT**HSDIQVK
IPI00169285	1	Hypothetical protein LOC196463	0.1	0.021	HPDAVAWA**NLT**NAIR
IPI00023673	1	Galectin-3 binding protein precursor	3.5	0.024	YKGL**NLT**EDTYKPR
IPI00218019	0.99	Splice Isoform 1 Of Basigin precursor	1.8	0.038	ILLTCSL**NDS**ATEVTGHR
IPI00009802	1	chondroitin sulfate proteoglycan 2 (versican) Splice Isoform 1 Of Versican core protein precursor	0.7	0.041	FE**NQT**GFPPPDSR
IPI00016112	1	Thyroid peroxidase PREDICTED: Melanoma associated gene	11.7	0.045	QGEHLS**NST**SAFSTR
IPI00339223	1	Splice Isoform 3 Of Fibronectin precursor	6.4	0.046	HEEGHML**NCT**CFGQGR
IPI00003813	1	Nectin-like protein 2	0.7	0.050	VS LT**NVS**ISDEGR
IPI00028931	0.98	Desmoglein 2 precursor	0.7	0.050	I**NAT**DADEPNTLNSK

[+] ProteinProphet probability score
*prostate over bladder ratio, fold change is indicated
** false positive rate<0.05 is considered to be significant for differential expression
*** putative glycosylation site is indicated in bold and underlined